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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 13661.10000	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/US04/26815	International filing date *(day/month/year)* 18 August 2004 (18.08.2004)	Priority date *(day/month/year)* 19 August 2003 (19.08.2003)
International Patent Classification (IPC) or national classification and IPC IPC(7): A01H 05/00, 01/00 and US Cl.: 800/317.3,278; 131/291,290		
Applicant 22ND CENTURY LIMITED, L.L.C.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 3 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 54 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____ , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04 April 2005 (04.04.2005)	05 July 2005 (05.07.2005)
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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US04/26815

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:
pages 1,5,7,8,12,14-25,28,30,31,33-36,40,42-47 and 53 as originally filed/furnished
pages* 2-4,6,9-11,13,26,27,29,32,37-39,41 and 48-52 received by this Authority on 25 April 2005 (25.04.2005)
pages* NONE received by this Authority on _____

☒ the claims:
pages NONE as originally filed/furnished
pages* NONE as amended (together with any statement) under Article 19
pages* 54-81 received by this Authority on 25 April 2005 (25.04.2005)
pages* NONE received by this Authority on _____

☒ the drawings:
pages 4 as originally filed/furnished
pages* 1-3,5 and 6 received by this Authority on 25 April 2005 (25.04.2005)
pages* NONE received by this Authority on _____

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing (*specify*): _____
☐ any table(s) related to the sequence listing (*specify*): _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing (*specify*): _____
☐ any table(s) related to the sequence listing (*specify*): _____

** If item 4 applies, some or all of those sheets may be marked "superseded."*

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US04/26815

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-148	YES
	Claims NONE	NO
Inventive Step (IS)	Claims 1-148	YES
	Claims NONE	NO
Industrial Applicability (IA)	Claims 1-148	YES
	Claims NONE	NO

2. Citations and Explanations (Rule 70.7)

Claims 1-148 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the claimed limitations.

Claims 1-148 meet the criteria set out in PCT Article 33(4), and thus meet industrial applicability because the subject matter claimed can be made or used in industry.

---------- NEW CITATIONS ----------

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global market value (The World Market for Tobacco Products, published by Euromonitor International, 2000 Edition, p. 2). In 2002, the worldwide market grew to 5.322 trillion cigarettes (Action on Smoking and Health, Factsheet No:18; January, 2004).

Considering the magnitude and growth rate of these numbers, it is clear that people will be smoking cigarettes for a long time to come. It is predicted that cigarette smoking could cause up to one billion premature deaths worldwide by the end of the 21st Century. Present statistics demonstrate that there is about one lung cancer death for every 3 million cigarettes consumed (Nature Cancer Reviews, Oct. 2001).

The ideal solution to this health care dilemma is for all cigarette smokers to quit. However, such a solution appears unrealistic. Prohibitionist anti-tobacco policies from the anti-tobacco lobby have been unsuccessful for the most part given the increasing worldwide consumption of tobacco products. These policies in the Western world, where they are most prevalent, have hardly reduced smoking rates over the last twenty years. A significant percentage of cigarette smokers have no desire to quit smoking. Even though tens of millions of people in the U.S. alone have quit smoking, many before the advent of the many forms of nicotine replacement therapies (NRTs), a segment of smokers is not successful in their attempts to quit. An effective strategy for reducing the adverse effects of cigarette smoking for these two groups has been deficient.

A recent report issued by the Institute of Medicine (IOM) of the National Academy of Sciences, at the request of the U.S. Food and Drug Administration, has laid the foundation for a potential remedy to the current impasse. The resulting 656-page report, titled Clearing the Smoke: Assessing The Science Base For Tobacco Harm Reduction (IOM Report), expresses an urgent public-health need for Potential Reduced-Exposure Products ("PREPs"), especially cigarettes (Institute of Medicine, Washington, DC: National Academy Press, 2001).

The first conclusion of the IOM Report is that: "For many diseases attributable to tobacco use, reducing risk of disease by reducing exposure to tobacco toxicants is feasible. This conclusion is based on studies demonstrating that for many diseases, reducing tobacco smoke exposure can result in decreased disease incidence

with complete abstinence providing the greatest benefit." (IOM Report Executive Summary, pg. 4).

PREPs are therefore a public-health policy necessity when considering all economic and political dynamics. The marketing and regulation of science-based PREPs needs to be included as part of any complete public-policy strategy on tobacco. The overall goal of reducing tobacco use, including reasonable tobacco marketing restrictions to adults, strict enforcement of laws banning sales and marketing to children, and education on the harmful effects of smoking, should go hand-in-hand with the availability of PREPs to consumers to reduce tobacco's overall toll on society.

Cigarette smoke is made up of two phases: a particulate phase, which is commonly called "tar" or total particulate matter, and a vapor phase, which contains gases and semi-volatile compounds. A common definition for "tar" is "nicotine-free dry smoke" or "nicotine-free dry particulate matter" (NFDPM). More specifically, "tar" is the total particulate matter isolated from smoke, excluding water and alkaloid compounds, including but not limited to nicotine. Approximately four-fifths of the weight of tobacco smoke is made up of ambient air, which includes carbon monoxide, carbon dioxide, water, hydrogen, methane, nitrogen and oxygen. The remaining one-fifth comprises the particulate phase and semi-volatile compounds. Tar makes up less than ten percent of the weight of cigarette smoke. Yet it is the tar component that contains the majority of the most harmful compounds.

Cigarette smoke is an extremely complex mixture of chemical compounds. Years of chemical analysis of cigarette smoke have demonstrated upwards of 6000 components (tar plus gases). Approximately 4800 compounds have been identified in the tar portion of cigarette smoke (Green and Rodgman, Recent Advances in Tobacco Science, 22:131-304, 1996). Analytical methods combined with sensitive biological assays have led to the identification of 69 carcinogens in tobacco smoke (The Changing Cigarette: Chemical Studies and Bioassays, Dietrich and Ilse Hoffman, Chapter 5, Smoking and Tobacco Control Monograph No. 13, NIH Pub. No. 02-5074, October 2001).

It has become clear to researchers, however, that not all components of cigarette smoke have equal toxicity. Notably, the first U.S. Surgeon General's report on smoking in 1964 came to the conclusion that nicotine was probably not toxic at the levels inhaled by smokers, with the implication that the source of the primary

pharmacologic reward to smokers was not of immediate concern (Gori, p. 3, Virtually Safe Cigarettes - Reviving an Opportunity Once Tragically Rejected, 2000). In fact, the Surgeon General's report indicated, "There is no acceptable evidence that prolonged exposure to nicotine creates either dangerous functional changes of an objective nature or degenerative diseases" (U.S. Surgeon General Report 1964, pg.74). Indeed, the U.S. Food and Drug Administration now allows the sale of nicotine patches and chewing gums as smoking cessation devices that may deliver more nicotine than a pack of cigarettes.

"Alkaloids" are complex, nitrogen-containing compounds that naturally occur in plants, and have pharmacological effects in humans and animals. "Nicotine" is the primary natural alkaloid in commercialized cigarette tobacco and accounts for about 90 percent of the alkaloid content in *Nicotiana tabacum*. Other major alkaloids in tobacco include cotinine, nornicotine, myosmine, nicotyrine, anabasine and anatabine (J. C. Leffingwell, Chapter 8 Leaf Chemistry, Tobacco: Production, Chemistry and Technology, pg. 275, 1999). Minor tobacco alkaloids include nicotine-n-oxide, N-methyl anatabine, N-methyl anabasine, pseudooxynicotine, 2,3 dipyridyl and others ("Biosynthesis and Metabolism of the Tobacco Alkaloids", Edward Leete in Alkaloids: Chemical and Biological Perspectives, Volume I, S. William Pelletier, Ed. 1983). Some of nicotine's common effects in humans are increased blood pressure and heart rate and improvements in concentration and short-term memory. Nicotine analogs and compounds are the subject of much recent research since they show promise in treating some diseases such as Alzheimer's and Parkinson's. Other tobacco alkaloids have similar but reduced activity compared to nicotine.

The most common measurements of cigarette smoke deliveries are reported as tar and nicotine. Tar and nicotine yields of cigarettes are shown in all consumer cigarette advertisements in the United States and numerous other countries. In many countries, yields (per cigarette) for tar, nicotine and even carbon monoxide are required to be printed on cigarette packaging. During the past several decades, cigarette design innovations have focused largely on tar and nicotine yield reductions, based on a belief embraced by the U.S. Surgeon General and the public health community that "less ought to be better" (See Figure 1).

In the United States, tar, nicotine, and carbon monoxide yields are obtained using the Federal Trade Commission (FTC) smoking-machine test method, which

or the filter plus eight millimeters. Both methods specify a 23-millimeter butt length for non-filter cigarettes.

- ISO defines the position of the ashtray at 20-60 millimeters below the cigarettes in the smoking machine. FTC does not specify a position.
- ISO specifies a two-piece snap together reusable filter holder. This filter holder contains the Cambridge pad and uses a synthetic rubber perforated washer to partly obstruct the butt end of the cigarette. The FTC method defines the use of a Cambridge filter pad but does not specify a filter pad holder assembly.
- The ISO method specifies airflow across the cigarettes at the cigarette level. FTC specifies the use of a monitor cigarette to adjust airflow.
- The ISO procedure defines the process of wiping the excess total particulate matter (TPM) out of the used filter holder. The inner surfaces of the filter holder are wiped with two separate quarters of an unused conditioned filter pad. The FTC method uses the backside (the side opposite of the trapped TPM) to wipe the inner surface of the filter holder.
- ISO specifies using 20 ml per Cambridge pad of extraction solution to analyze nicotine and water in TPM. The FTC procedure specifies 10 ml per Cambridge pad.
- ISO defines the internal standards for the gas chromatographic determination of nicotine and water. The FTC procedure does not specify the internal standards.

These differences typically result in slightly lower measured deliveries for the ISO Method versus the FTC Method. The measured values between FTC and ISO methods are within the detection limits of the test or about no greater than 0.4 mg tar and about 0.04 mg nicotine for cigarettes that yield over about 10 mg.

The primary criticism of the FTC/ISO smoking-machine test methods ("FTC/ISO Method" or "FTC or ISO Method") is that they do not accurately predict an individual smoker's level of exposure to tar, nicotine or carbon monoxide from smoking a particular cigarette (National Cancer Institute Smoking and Tobacco Control Monograph 13, "Risks Associated with Smoking Cigarettes with Low Machine-measured Yields of Tar and Nicotine). These methods obtain test results under standardized conditions. However, an individual's smoking behavior may, and

to make cigarettes because losses occur during the curing, storing and manufacturing processes.

"Curing" is the aging process that reduces moisture and brings about the destruction of chlorophyll giving tobacco leaves a golden color and by which starch is converted to sugar. Cured tobacco therefore has a higher reducing sugar content and a lower starch content compared to harvested green leaf. "Flue-cured tobacco" refers to a method of drying tobacco plants in a ventilated barn with heat and is characterized by a unique color, high reducing sugar content, medium to heavy in body and exceptionally smooth smoking properties (Bacon, E.W., Wenger, R. & Bullock, J.F. (1952), Chemical changes in tobacco during flue-curing, Ind. Eng. Chem., 44, 292).

It is known that by varying the design of any of the components of the cigarette rod, as discussed above—for virtually all commercialized tobacco fillers—the levels of tar and nicotine that are measured by the FTC/ISO Method can be varied for a filtered cigarette from approximately 1 mg of tar and 0.05 mg of nicotine to approximately 20 mg of tar and 1.8 mg of nicotine.

When cigarettes are designed to be "lighter," tar, nicotine, and carbon monoxide levels, as measured by the FTC/ISO Method, are reduced at slightly different rates. Nevertheless, the level of tar and carbon monoxide are not reduced by any sizable percentage without a corresponding reduction in the level of nicotine by approximately the same percentage and vice versa. Even though tar and nicotine yields per the FTC/ISO Method have been reduced over the last fifty years, the "tar-to-nicotine yield ratio" ("TNR") of cigarettes has remained quite stable, as indicated in Figure 1 and Figure 2.

The term "cigarette" as used herein is defined as the "rod" plus the "filler". The cigarette "rod" includes the cigarette paper, filter, plug wrap (used to contain filtration materials), tipping paper that holds the cigarette paper (including the filler) to the filter, and all glues that hold these components together. The only components of the rod of a "non-filter cigarette" are the cigarette paper and glue that seals it. The "filler" includes (1) all tobaccos, including but not limited to reconstituted and expanded tobacco, (2) non-tobacco substitutes (including but not limited to herbs, non-tobacco plant materials and other spices that may accompany tobaccos rolled within the cigarette paper), (3) casings, (4) flavorings, and (5) all other additives (that are mixed into tobaccos and substitutes and rolled into the cigarette). The term

"cigarette" as used herein is also defined as (A) any roll of tobacco wrapped in paper or any other substance not containing tobacco, and (B) any roll of tobacco wrapped in any substance containing tobacco which, because of its appearance, the type of tobacco used in the filler, or its packaging and labeling, is likely to be offered to, or purchased by, consumers as a cigarette described in subparagraph (A) (1967 Federal Cigarette Labeling And Advertising Act, U.S. FTC).

The terms "non-filter rod," "full-flavor rod," "light rod" and "ultra-light rod" as used herein are defined as a non-filter cigarette minus its filler, a full-flavor cigarette minus its filler, a light cigarette minus its filler, and an ultra-light cigarette minus its filler, respectively.

As used herein, the "tar-to-nicotine yield ratio" or "TNR" of a cigarette is calculated by dividing the tar yield by the nicotine yield, both of such yields being measured by the FTC or ISO Method.

Cigarette brands in the United States and throughout most of the world are differentiated by categories such as full-flavor, lights, and ultra-lights. These designations usually appear on cigarette packs and advertising. Such categories convey cigarette strength, which is a function of the level of tar and nicotine measured by the FTC or ISO Method. Stronger-tasting or full-flavor cigarettes have higher tar and nicotine yields. The categories or "strength" of cigarettes that are generally recognized in the United States as per the FTC Method are the following:

- "full-flavor cigarette" (15 mg or more tar per cigarette)
- "light cigarette" (7 to 14 mg tar per cigarette)
- "ultra-light cigarette" (6 mg or less tar per cigarette).

Consumer decisions on whether to smoke full-flavor versus light cigarettes based solely on tar and nicotine levels derived from the FTC/ISO Method are problematic ("Public Understanding of Risk and Reasons for Smoking Low-Yield Products", Neil Weinstein, NCI Monograph 13, Chapter 6). Since humans and smoking machines smoke cigarettes differently, the consumer may have high expectations for a cigarette reported to have low-tar ("Consumer Perception of Cigarette Yields: Is the Message Relevant?", Gio Gori, Regulatory Toxicology and Pharmacology volume 12, 64-68, 1990). The smoker should not mistakenly believe that switching from full-flavor cigarettes (Marlboro® full-flavor produce 15 mg tar

and 1.1 mg nicotine) to light cigarettes (Marlboro® lights yield 11 mg tar and 0.8 mg nicotine) will necessarily reduce the risks associated with smoking.

When light cigarette smokers are compared to full-flavor cigarette smokers (and ultra-light smokers compared to light smokers and ultra-light smokers compared to full-flavor smokers), and/or when an individual smoker who usually smokes full-flavor or light cigarettes switches to reduced yield cigarettes, or occasionally smokes reduced yield cigarettes, some or all of the following smoking behaviors may occur to some extent:

- More puffs taken per cigarette;
- Larger individual puffs or puff volume (e.g., 55 ml of smoke may be consumed versus 35 ml);
- Variation in the duration of individual puffs (e.g., 4 seconds versus 2 seconds), therefore producing hotter cone temperatures, which have been associated with increased smoke mutagenicity*;
- Holding smoke in the lungs for a longer duration before exhaling;
- Deeper inhalation into the lungs;
- Light cigarette smokers may block filter vent holes with fingers and lips; and
- Smoking more cigarettes over a given period of time

*("Effect of pyrolysis temperature on the mutagenicity of tobacco smoke condensate," White, J.L., et al., Food and Chemical Toxicology 39, pg. 499-505 (2001)). As stated in the IOM Report: "In order to maintain the desired intake of nicotine, many smokers who changed to low-yield products also changed the way they smoked in the manner previously described. Thus, their exposure to tobacco toxicants is higher than would have been predicted by standardized assays and people who have continued to use these products have not significantly reduced their disease risk by switching to them" (IOM Report p.2).

Differences in smoking behavior observed among smokers of full-flavor, light, and ultra-light cigarettes have been collectively called "compensation" ("Compensatory Smoking of Low Yield Cigarettes", Neal Benowitz, NCI Monograph 13). "Compensation" is smoking more intensively due to the reduced presence of nicotine in tobacco smoke. Smokers compensate by smoking lower-yield cigarettes

demonstrates that in 1950 the average TNR was about 14.44 (about 39 mg tar/about 2.7 mg nicotine). These numbers demonstrate that the average TNRs of American cigarettes from about 1950 to the present have been fairly consistent.

The 1998 FTC Report was released in 2000, covering the cigarette brands of 1998, and was the last year that the FTC chose to publicly release tar, nicotine and carbon monoxide yields from cigarettes. Out of the 1294 cigarette brand styles evaluated, only 3 have a calculated TNR below 8. In fact, only a total of 8 brand styles have calculated TNRs of less than 10. These consist of 1 Rothmans®, 3 Canadian Players®, 2 Old Gold®, 1 Now®, and 1 Carlton®. While only the tar, nicotine and carbon monoxide numbers were listed in the report, TNRs can be easily calculated from these numbers by dividing the yield of tar by the yield of nicotine. Two other Carlton® brand styles have yields of <0.5 tar and 0.1 nicotine. Since the actual raw numbers of the tar yields can not be determined and since there is an enormous impact due to rounding at these levels, the numbers for these 2 brand styles do not appear to reflect the prior art.

2 of the 3 brand styles with TNR's of less than 8 (Carlton® 100 filter soft pack and Now® king filter soft pack) were reported in the 1998 FTC Report to yield 1 mg tar and 0.2 mg nicotine, thereby having a TNR of 5. However, the TNRs of these 2 brand styles are mainly due to the nature of rounding small numbers. From the 1998 FTC report, "Tar and carbon monoxide ratings are rounded to the nearest milligram (mg.); those with 0.5 mg or greater are rounded up, while those with 0.4 mg or less are rounded down. The nicotine figures are rounded to the nearest tenth of a milligram. Those with 0.05 mg or greater are rounded up; those with 0.04 mg or less are rounded down." Therefore, an ultra-light cigarette delivering 1.4 mg of tar and 0.15 mg nicotine would be reported as 1 mg tar and 0.2 mg nicotine (TNR of 5), even though the actual TNR would equal 9.33. Cigarettes delivering 1 mg of tar and 0.1 mg nicotine have low consumer acceptability due to smoke thinness (lack of taste) and too little draw resistance. In 2000, the market share of cigarette brand styles that yielded 1-3 mg tar pursuant to the FTC Method had a U.S. market share of only 1.3 percent. Ninety-two percent of the brand styles yielding 3 mg tar or less tar printed their FTC tar and nicotine ratings on their packs. This contrasts to brand styles that yielded 12 or more mg tar, in which only .01 of one percent printed their FTC tar and nicotine rating on their packs (FTC Cigarette Report for 2000, 2002, p. 15).

This is because such cigarettes reduce tar and nicotine yields concurrently and at about the same rate.

The present invention, in conjunction with different levels of filtration and/or smoke dilution, provides for increasing the nicotine content of the cigarette's filler, either genetically within the tobacco plant, or by adding nicotine to the filler, thereby allowing the cigarette's nicotine delivery to the smoker to be maintained, while preferably decreasing the cigarette's tar delivery.

As used herein, "increased-nicotine transgenic plant" means a recombinant (or "transgenic") tobacco plant that contains a higher nicotine content than the non-transgenic "parent" (or unmodified "control") plant from which the transgenic plant is produced.

As used herein, "increased-alkaloid transgenic plant" means a recombinant (or "transgenic") tobacco plant that contains a higher total alkaloid content than the non-transgenic "parent" (or unmodified "control") plant from which the transgenic plant is produced.

As used herein, "reduced-nicotine transgenic plant" means a recombinant (or "transgenic") tobacco plant that contains less than half, preferably less than 25%, and more preferably less than 20% or less than 10%, of the nicotine content of the non-transgenic "parent" (or unmodified "control") plant from which the transgenic plant is produced. It will be appreciated that some small level of residual nicotine, on the order of at least 1% or 5% as compared to the corresponding unmodified control plant, may remain in such transgenic plants used to carry out the present invention.

As used herein, "nicotine" (C10H14N2) includes analogs of nicotine (unless nicotine is referenced to total alkaloid(s)), nicotine's two isomers, synthesized nicotine, and nicotine salts of organic acids.

Plants for use in the present methods are species of the genus *Nicotiana*, or tobacco, including but not limited to *Nicotiana tabacum, Nicotiana rustica, Nicotiana glauca, Nicotiana excelsior, Nicotiana benthamiana, Nicotiana sylvestris, Nicotiana clevelandii* and *Nicotiana attenuata*. As used herein, "tobacco" means and encompasses any plant, species, crosses, or hybrids of the genus *Nicotiana*. Any strain or variety of tobacco may be used. Such tobacco plants are genetically modified to either increase or reduce the nicotine content, depending on intent, thereof as

discussed in greater detail below. The term "plant" includes physical and chemical portions thereof, such as plant parts and plant extracts, hydrolysates, etc.

As used herein a "low TNR cigarette" or "low tar-to-nicotine yield ratio cigarette" of the present invention means a cigarette that contains an increased-nicotine recombinant tobacco plant or plant portion, including but not limited to nicotine from such plant or plant portions.

1. Low TNR Cigarettes (with increased-nicotine transgenic tobacco); Production of Novel Tobacco Varieties; and More Acceptable Low TNR Cigarettes

Increased-nicotine transgenic tobacco plants used to carry out the first aspect of the present invention are, in general, recombinant tobacco plants that contain and express a heterologous nucleotide, the expression of which up-regulates an enzyme (such as arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS)) in the plant, and thereby increases the production of nicotine in the plant. Suitable recombinant plants are disclosed in M. Conkling et al., PCT Application WO98/56923 (published Dec. 17, 1998) and in M. Timko, PCT Application WO00/67558 (published Nov. 16, 2000). In general, the heterologous nucleotide comprises at least a segment of a nucleic acid encoding the enzyme to be up-regulated.

In this embodiment, increased-nicotine tobacco is incorporated into the filler of a cigarette to achieve a desired low TNR cigarette. With the combination of a full-flavor rod, preferably a light rod, and more preferably an ultra-light rod, increased-nicotine tobacco (that may include blends of conventional tobacco) cigarettes can now efficiently deliver the smoker's desired amount of nicotine per cigarette, while delivering less tar and harmful gases. The inventive low TNR cigarette is also achievable by using a non-filter rod, since smokers of non-filter cigarettes will still have the increased presence of nicotine, which in some cases will reduce inhalation of tar and harmful gases. A lowered TNR cigarette is therefore a major goal of the inventive PREP.

One specific embodiment utilizes an increased-nicotine recombinant plant that has increased quinolate phosphoribosyl transferase (QPRTase) expression relative to a non-transformed control plant, such recombinant plant comprising recombinant plant

In another embodiment, the same plant or cell may contain at least one recombinant nucleic acid that up-regulates an enzyme in the nicotine or alkaloid synthesis pathway, while also containing at least one recombinant nucleic acid that down-regulates an enzyme in the nicotine or alkaloid synthesis pathway. As an example, if PMT is up-regulated, and QPT is down-regulated, the nicotine to total alkaloid ratio will increase. This ratio is preferably as close to one as possible for advantages for the prevention of the formation of NAT, NAB and possibly other TSNAs. See Figure 5 and Figure 6.

In another embodiment, the present invention utilizes a increased-nicotine recombinant plant that has both increased QPRT and increased PMT expression relative to a non-transformed control plant, such recombinant plant comprising recombinant plant cells containing: (i) a first exogenous DNA construct comprising, in the 5' to 3' direction, a promoter operable in such plant cell and a heterologous DNA encoding at least a segment of a plant quinolate phosphoribosyl transferase mRNA, such heterologous DNA operably associated with such promoter; and (ii) a second exogenous DNA construct comprising, in the 5' to 3' direction, a promoter operable in such plant cell and a heterologous DNA encoding at least a segment of a plant PMT mRNA, such heterologous DNA operably associated with such promoter, such plant exhibiting increased QPRT and increased PMT expression compared to a non-transformed control plant and increased-nicotine content as compared to a non-transformed control plant. As used herein, QPT, QPRT, and QPRTase are used interchangeably. PMT and PMTase are also used interchangeably.

Examples of recombinant plants that may be used to carry out these embodiments include, but are not limited to, known plants transformed with DNA encoding the tobacco quinolate phosphoribosyl transferase gene (NtQPT1) (see, *e.g.*, PCT Application WO98/56923 by Conkling et al.); DNA encoding tobacco putrescine N-methyltransferase, such as PMT1, PMT2, PMT3 and PMT4; DNA encoding tobacco arginine decarboxylase, such as ADC1 and ADC2; DNA encoding tobacco omithine decarboxylase (ODC); DNA encoding tobacco S-adenosylmethionine synthetase (SAMS); DNA encoding tobacco NADH dehydrogenase; and DNA encoding tobacco phosphoribosylanthranilate isomerase (PRAI) (which are known and described in PCT Application WO 00/67558 by M. Timko et al.).

content of 15.7 percent, 15.5 percent, and 15.2 percent, respectively (2000 Official Flue-Cured Variety Test at the University of Georgia, Tifton).

In this regard, the terms "cultivar" and "variety" are used synonymously to refer to a group of plants within the species, *N. tabacum,* that share certain constant characteristics separating them from the typical form and from other possible varieties within that species. While possessing at least one distinctive trait, a variety also may be characterized by a substantial amount of overall variation between individuals within the variety, based primarily on the Mendelian segregation of traits among the progeny of succeeding generations. A "line," as distinguished from a "variety," denotes a group of plants which display less variation between individuals, generally (although not exclusively) by virtue of several generations of self-pollination. In addition, a "line" is defined, for the purpose of the present invention, sufficiently broadly to include a group of plants vegetatively propagated from a single parent plant, using tissue culture techniques. The use of such lines to develop new hybrids is described in U.S. Pat. Nos. 4,326,358 and 4,381,624.

A "nicotine buffer" helps maintain the pH of cigarette smoke. As nicotine is a major volatile base present in cigarette smoke, smoke pH imparts an important role in sensory perception. Sugar acts as a nicotine buffer, reducing any harshness from the increased nicotine; hence, a high sugar content is beneficial, whether the sugar is natural to the tobacco plant or is added, *e.g.*, as high fructose corn syrup, sucrose, invert sugar, licorice extract, carob bean and extract, and cocoa and cocoa extracts during tobacco processing.

"Reducing sugar(s)" are any sugar (monosaccharide or polysaccharide) that has a free or potentially free aldehdye or ketone group. Glucose and fructose act as nicotine buffers in cigarette smoke by reducing smoke pH and effectively reducing the amount of "free" unprotonated nicotine. Reducing sugars balance smoke flavor, for example, by modifying the sensory impact of nicotine and other tobacco alkaloids. Generally, there is an inverse relationship between sugar content and alkaloid content across tobacco varieties, within the same variety, and within the same plant line caused by planting conditions. For example, the lower the nitrogen is in tobacco's soil, the lower the nicotine levels but the higher sugar levels. Increased rain produces lower nicotine levels and higher sugar levels.

It will be appreciated that tobacco plants of the present invention may not be "transgenic," i.e. may not contain nucleic acid sequences from other organisms incorporated in their genome, yet the levels of nicotine, sugars or fatty acids may be modified by producing plants or plant cells through targeted mutagenesis of specific nucleic acid sequences by introduction of nucleic acids that induce DNA repair or recombination (Beetham et al., 1999; Zhu et al., 2002; WO 03/013226), or introduction of modified viruses which may produce similar end results as "increased-nicotine transgenic plants," "reduced-nicotine transgenic plants," "increased-sugar transgenic plants," and "increased-fatty acid transgenic plants" described herein. A "Precise Breeding" method (U.S. Pub. No. 20040107455), in which only nucleic acid sequences derived from the target species or sexually compatible species are introduced into the genome of the target plant, may be used to produce plants that are "increased-nicotine plants," "reduced-nicotine plants," "increased-sugar plants," and "increased-fatty acid plants" described herein.

2. Low TNR Cigarettes (by adding nicotine from transgenic increased-nicotine plants)

A second aspect of the present invention is a method of adding nicotine or a nicotine-containing fraction extracted from an increased-nicotine transgenic plant, as described above, to conventional tobacco or reduced-nicotine tobacco at the required levels.

U.S. Patents 4,830,028, 4,836,224 and 5,031,646 describe modification of cigarette filler by addition of organic acid salts of nicotine. In particular, they address addition of nicotine in this manner in order to decrease the tar-to-nicotine yield ratio. A review of the Brown and Williamson Tobacco Company research in the Journal of the American Medical Association (volume 274, No. 3, p.228, 1995) describes Project Ariel covered by U.S. Patents 3,258,015 and 3,356,094 (Battelle). These patents describe an aerosol with nicotine added in such a manner capable of reducing the tar-to-nicotine yield ratio of the inventive cigarettes to one quarter of that of conventional cigarettes.

Nicotine-containing fractions, nicotine, or nicotine salts of organic acids obtained from increased-nicotine transgenic plants are added to conventional tobacco or reduced-nicotine transgenic plants by spraying or using any other method to one skilled in the art of tobacco processing and additive applications, with or without

propylene glycol or any other solvent, or water, for dissolution, onto whole leaf or cut-rag tobacco. The amount of nicotine that has to be added in designing a low TNR cigarette is a function of the following: (1) the nicotine content and type of tobacco blend being used - American blend cut rag tobacco generally contains approximately 2 to 2.5 percent nicotine, (2) the specifications of the cigarette rod, with consideration of ventilation or porosity properties, (3) the desired TNR of the cigarette, and (4) the specific tar yield desired.

For example, let's assume a cigarette manufacturer wants to create a low TNR cigarette that yields 8 mg tar and the same 1.2 mg nicotine that its full flavor brand style yields as per the FTC/ISO Method. It's full flavor brand style yields 16 mg tar so the goal is for the novel brand style's TNR to be half that of the full-flavor brand style. It is also desired to use the same filler and then add nicotine to create the low TNR cigarette.

The amount of nicotine that the manufacturer would initially add during product development for a yield 8 mg tar and of 1.2 mg nicotine is about twice as much as the nicotine content of its filler for its full flavor brand. It will be appreciated by one skilled in the art that the extra ventilation of the light rod will reduce the tar yield at a slightly higher rate than the nicotine yield so that doubling the nicotine of the filler may be slightly too much. However, some nicotine may be lost during the tobacco processing and cigarette manufacturing processes, so doubling the nicotine could be justified. The impact from the duration of such tobacco being stored after the addition of nicotine, but before cigarette manufacturing, must also be evaluated. Since the scale and manner of these processes are different for every manufacturer, it will be appreciated that some trial and error may be necessary.

A light rod that usually yields 8 mg of tar per cigarette can initially be chosen by the manufacturer. The result that is desired is a cigarette that yields 8 mg tar and 1.2 mg nicotine, thereby cutting tar more than half yet keeping nicotine yield the same as most full-flavor cigarettes.

A selected amount of tobacco, after adding nicotine, or nicotine salts of organic acids, at about 13 percent moisture is placed into the hopper of a cigarette-making machine. The cigarette-making machine then passes the rolled cigarettes to another machine that puts the filter on the unfiltered rolled cigarette. This step is skipped if non-filter cigarettes are being produced.

The finished cigarettes are then tested using the FTC/ISO Method so that end results can be evaluated. If the cigarette yields 8 mg tar and 1.2 mg nicotine, (a TNR of 6.66) and all of the above considerations have been accounted for, then the development has been completed. If the cigarette is being rated harsh in focus groups due to the extra nicotine, then additives should be added to the filler to help alleviate the problem.

Minor adjustments can be made if the cigarettes manufactured yield more or less than the desired yields. These include changing one of the components of the cigarette rod by varying the types of filters, cigarette paper, plug wraps, the tipping paper (that holds the cigarette rod to the filter), the ventilation holes, or variations in combination. For example, if the cigarette is yielding 9 mg of tar and 1.3 mg nicotine, the filtration and/or dilution is slightly increased to reduce both tar and nicotine yields in a very similar proportion. This is usually achieved by specifying the size and quantity of the ventilation holes and the porosity of the filter plug wrap to achieve the desired yield. If the tar yield is on target but the nicotine yield is higher or lower than desired, then the levels of added nicotine can be adjusted accordingly.

Most brands of cigarettes have from about 10 percent of reconstituted tobacco in premium brands to about 30% in discount brands in the filler of the cigarette even though it is not a necessary part of the filler. Another embodiment of the present invention is to add nicotine to the reconstituted portion of the cigarette filler in order to give the cigarettes a lower TNR. Adjustments in the amount of nicotine to add to low TNR cigarettes is another variable which can be accomplished by the manufacturer. Therefore, the nicotine content of the filler is a function of the nicotine content of the tobacco plus the nicotine content of any reconstituted tobacco in the filler, including increased-nicotine recon and/or reduced-nicotine recon, or both. Any combination of the three can be utilized.

A 1975 paper from the Philip Morris Document Website (Bates 2056140416, Titled "Low Delivery Cigarettes and Increased Nicotine/Tar Ratios, A Replication") describes results from a taste panel of cigarettes with increased nicotine/tar ratios. It was found that a cigarette with a 10 mg tar delivery and a nicotine/tar ratio of 0.09 (TNR equivalent of 11) was equal in acceptability and strength to the Marlboro® full-

is to use reduced-nicotine transgenic tobacco and add extracted nicotine, nicotine salts derived from organic acids (derived from conventional or genetically modified increased-nicotine tobacco), or synthesized nicotine, in free base or combined with organic acids, to in order to create a cigarette or other tobacco products with virtually no nitrosamines or minor alkaloids, yet one that yields a conventional amount of nicotine. This method is utilized with genetically modified reduced-nicotine tobacco by adding only enough nicotine to provide the same yield as conventional cigarettes with the FTC/ISO Method, *e.g.*, from 0.05 to 1.5 mg per cigarette.

Genetically altering the alkaloid content in tobacco has been carried out by altering quinolate phosphoribosyl transferase (QPT) and putrescine methyltransferase (PMT). Figure 4 shows how alkaloids including Nor-Nicotine have been reduced by reducing QPRTase in a burley variety to create a low alkaloid transgenic variety named Vector 21-41. This tobacco variety contains very low levels of TSNAs. The Vector 21-41 variety is protected by the Plant Variety Protection Office and its Plant Variety Protection Number is 200100039.

"Transformed root lines were produced that contained markedly reduced PMT activity, with a concomitant reduction in nicotine content compared to controls" (Yupynn Chintapakorn and John D. Hamill, Plant Molecular Biology 53: 87–105, 2003, 2003 Kluwer Academic Publishers). Nornicotine is negatively correlated with tobacco quality and cigarette taste ("Natural Tobacco Flavor", Roberts, D.L., Recent Advances in Tobacco Science, 14, pg. 49-81, 1988). A cigarette product produced with nicotine as the only alkaloid should be highly acceptable on this basis alone.

Reduced-nicotine tobacco plants used to carry out the present invention are, in general, recombinant tobacco plants that contains and express a heterologous nucleotide, the expression of which heterologous nucleotide down-regulates an enzyme such as quinolate phosphoribosyl transferase (QPRTase), putrescine methyltransferase (PMTase), arginine decarboxylase, omithine decarboxylase, S-adenosylmethionine synthetase, NADH dehydrogenase, or phosphoribosylanthranilate isomerase (PRAI) in the plant, and thereby reduces the production of nicotine in the plant. Suitable recombinant plants are disclosed in M. Conkling et al., PCT Application WO98/56923 (published Dec. 17, 1998) and in M. Timko, PCT Application WO00/67558 (published Nov. 16, 2000). In general, the heterologous

invention is to utilize reduced-nicotine tobacco and then add nicotine so that a conventional amount of nicotine is present for the following products: cigar filler or wrapper, roll-your-own tobacco for cigarettes, pipe tobacco, chewing tobacco, snuff, reconstituted tobacco, and all other versions of smokeless tobacco. The advantages being that these products are extremely low in TSNAs and/or minor tobacco alkaloids.

4. Production of Improved Expanded or Puffed Tobacco Using Increased-Nicotine Transgenic Tobacco

More than 150 patents have been issued related to tobacco expansion (*e.g.*, U.S. Patent Number 3,991,772). The expansion process gives greater filling power to the tobacco so less tobacco weight in used in the cigarette. An advantage of using expanded tobacco is reduced tar delivery. Expanded tobacco is particularly useful in making low-tar delivery cigarettes. Carlton® cigarettes, which has had claims that it is the lowest tar and nicotine delivery cigarette, is reportedly made with a very large percentage of expanded tobacco. However, use of expanded tobacco also results in reduced nicotine delivery, which may result in compensation.

The main benefit of expanded increased-nicotine transgenic tobacco is that such tobacco provides reduced tar delivery while about maintaining nicotine delivery, resulting in a cigarette with reduced tar delivery and a reduced TNR. For example, a tobacco blend of a cigarette incorporating increased-nicotine transgenic tobacco may deliver 16 mg tar and 2.0 mg of nicotine (TNR of 8). Expanding such transgenic tobacco 100% would give the tobacco filler less weight but it would occupy the same volume in such cigarette, thereby reducing the TNR. The TNR of this cigarette would be less than 8, without filter ventilation.

Any method for expansion of tobacco known in the art may be used in the present invention. The most common method used today incorporates liquid carbon dioxide (U.S. Patent Nos. 4,340,073 and 4,336,814). Liquid propane has also been used for making commercial cigarettes, predominantly in Europe (U.S. Patent No. 4,531,529). Liquid propane offers advantages over carbon dioxide since higher degrees of expansion are possible, in the range of 200%. Under pressure, the liquid carbon dioxide (or liquid propane) permeates the tobacco cell structure. When the tobacco is rapidly heated the carbon dioxide (or liquid propane) expands the cell back to its pre-cured size.

It is another embodiment of the present invention to utilize increased-nicotine transgenic tobacco, preferably tobacco that was created from a high-sugar and/or high fatty acid background and create expanded tobacco from such transgenic tobacco to produce a low TNR cigarette.

It is another embodiment to utilize deproteinized tobacco, preferably extracted from reduced-nicotine transgenic tobacco, and create expanded tobacco from such deproteinized tobacco. A cigarette containing deproteinized expanded tobacco and increased-nicotine transgenic tobacco is thereby produced.

5. Production of Reconstituted Tobacco

It is another embodiment of the present invention to produce reduced-exposure tobacco products, which may include low TNR cigarettes, by utilizing the previous inventions above, deproteinized tobacco fiber, and freeze dried tobacco in any combination and in conjunction with reconstituted tobacco.

The process to produce sheets of reconstituted tobacco ("recon") began during the 1950s. U.S. Patent Nos. that describe such processes include: 3,499,454, 4,182,349, 4,962,774, and 6,761,175. Recon is traditionally produced from tobacco stems and/or smaller leaf particles that closely resembles a typical paper making process. The tar and nicotine yields of reconstituted tobacco are lower than those from equivalent quantities of whole tobacco leaf. This process entails processing the various tobacco portions that are to be made into Recon. After the Recon sheets are produced they are cut into a size and shape that resembles cut rag tobacco made from whole leaf tobacco. This cut recon then gets mixed with cut-rag tobacco and is ready for cigarette making.

Cigarettes can be manufactured with all recon, no recon, or any combination thereof. Most major brands have at least 10% of Recon in the Filler.

The main benefit of increased-nicotine transgenic tobacco used for recon is that such tobacco will reduce the tar yield of cigarettes, while about maintaining nicotine yield.

It is another embodiment of the present invention to add nicotine, or nicotine salts, to produce recon, which is made from reduced-nicotine transgenic tobacco or any non-tobacco plant material including but not limited to herbal blends so that when such reconstituted sheet is burned it yields substantially less tobacco-specific

nitrosamines and other carcinogens produced from conventional cigarettes, yet satisfactory amounts of nicotine are present.

It is another embodiment of the present invention to utilize increased-nicotine transgenic tobacco, preferably such tobacco that was created from a high-sugar and/or high fatty acid background and create recon from such transgenic tobacco to produce a low TNR cigarette. Another embodiment increases the sugar content, the fatty acid content, or both of the recon during processing.

Recon from tobacco fiber

Patents describing processes of removing proteins from tobacco, thereby creating "deproteinized tobacco fiber" are described in U.S. Patent Nos. 4,289,147 and 4,347,324. Tobacco fiber is a major byproduct after removing protein. The fibrous remains from deproteinized tobacco can be included in any percentage as an ingredient of reconstituted tobacco. Cigarettes made from deproteinized tobacco have a different taste than conventional cigarettes. However, appropriate amounts of additives, including flavorings and nicotine, could be added to help alleviate this taste deficiency.

Cigarettes containing deproteinized tobacco have a significant advantage over conventional cigarettes since they would produce reduced levels of carcinogens and harmful combustion products. "A 71% reduction in protein content of a flue-cured tobacco sheet resulted in an 81% reduction in the TA98 Ames mutagenicity" of the pyrolytic condensate (Clapp, W.L., et al., "Reduction in Ames Salmonella mutagenicity of cigarette mainstream smoke condensate by tobacco protein removal", Mutation Research, 446, pg 167-174, 1999). Previous research in this area had determined that tobacco leaf protein might be the principal precursor of mutagens in tobacco smoke condensate (Matsumoto, et al., "Mutagenicities of the pyrolysis of peptides and proteins", Mutation Research, 56, pg 281-288, 1978).

Extracting tobacco fiber from genetically modified reduced-nicotine tobacco (*e.g.*, Vector 21-41) effectively eliminates virtually all carcinogenic TSNAs from such tobacco, since nitrosamines require relatively high concentrations of nicotine and other alkaloids to form at detectable levels. See FIGURE 4

Therefore, it is advantageous to utilize reduced-nicotine tobacco in reduced-exposure cigarettes or other tobacco products to further reduce nitrosamines. Nicotine

is either left out or introduced later in the process, which can also be in the form of nicotine salts.

Polycyclic aromatic hydrocarbons (PAHs) are formed from high temperature pyrolysis of amino acids, sugars, paraffins, terpenes, phytosterols, celluloses and other components of tobacco. Most of these components are greatly reduced in tobacco fiber, effectively reducing formation of PAHs. Catechols and phenols, recognized carcinogenic co-factors in cigarette smoke, would also be reduced since low levels of soluble sugar are present in tobacco fiber.

Harmful gas phase compounds such as hydrogen cyanide, nitrogen oxides, and carbon monoxide are also reduced when cigarettes containing only tobacco fiber are smoked compared to cigarettes made with whole-leaf tobacco. Hydrogen cyanide is formed from burning proteins and chlorophyll. Nitrogen oxides are formed from burning soluble protein, chlorophyll, nitrates, and alkaloids. These components would not be present in significant amounts in deproteinized tobacco. Tobacco fiber has approximately 85 percent less starches and cellulosic material thus reducing the major pyrolytic precursors of carbon monoxide.

It is another embodiment of the present invention to produce reconstituted tobacco that includes extracted tobacco fiber derived from conventional tobacco, reduced-nicotine transgenic tobacco, or increased-nicotine transgenic tobacco.

Recon from freeze-dried tobacco

If the tobacco curing process is circumvented, virtually no TSNAs will be present in traditional tobacco products such as cigarettes, cigar filler or wrapper, roll-your-own tobacco for cigarettes, pipe tobacco, chewing tobacco, snuff, reconstituted tobacco and other preparations made with freeze-dried tobacco would contain virtually no TSNAs since traditional curing processes are eliminated.

Another embodiment of the present invention is the virtual elimination of TSNAs through processing freshly harvested tobacco using lyophilization. This is accomplished by processing freshly harvested tobacco through freeze-drying units located near tobacco farms. Tobacco processed in this manner may be grown in a traditional fashion with spacing of plants or in a biomass setting. In addition to the economic advantages of eliminating the costs associated with the curing process, the tobacco can now be grown in a biomass fashion that can create hundreds of thousands of pounds of fresh tobacco per acre.

By growing tobacco in a biomass setting and immediately freeze drying the fresh tobacco for cigarettes, roll-your-own-tobacco, pipe tobacco, cigar filler or wrapper, chewing tobacco, snuff, and other versions of smokeless tobacco, labor is reduced not only by eliminating the transplant of each plant from greenhouse to the field but also by eliminating traditional harvesting and curing of the tobacco. Also, farmland needed for this purpose is greatly reduced. The yield of tobacco from one acre of tobacco grown in biomass is equivalent to approximately 100 acres of tobacco grown in a traditional manner.

"Tobacco biomass" is achieved by direct sowing an acre of land with copious quantities of tobacco seed within a few inches of each other in the field. Unlike tobacco planted with traditional spacing, individual plants can no longer be differentiated when tobacco is planted in a biomass fashion. An acre of tobacco biomass has the appearance of a continuous, dense, green carpet. U.S. Pub. Pat. App. No. 20020197688 describes such methods.

Lyophilization removes most of the water (~80%) from the weight of fresh harvested tobacco biomass. The result is Freeze Dried Tobacco ("FDT"). FDT is easily pulverized into fine particles suitable for processing into reconstituted tobacco sheet (recon). This recon can be cut and made into any type of tobacco product such as filler for cigarettes, roll-your-own-tobacco, pipe tobacco, cigar filler or wrapper, chewing tobacco, snuff, and other forms of smokeless tobacco. Flavorings and additives, including sugars, can be incorporated into the recon process.

Such recon can be made from 100 percent FDT or in any proportion that consumers prefer. The lyophilization process may have adverse affects on the taste of such tobacco products. Therefore, FDT can even be mixed in any percentage with traditional pulverized, cured tobacco so that the mixture can be made into reconstituted tobacco. Alternatively, FDT can be mixed in any percentages with any form of traditional tobacco conducive for manufacturing cigarettes, roll-your-own-tobacco, pipe tobacco, and cigar filler or wrapper, chewing tobacco, snuff and other versions of smokeless tobacco in order to satisfy the tastes of the mass market.

Another embodiment of the present invention is to use genetically modified reduced-nicotine tobacco for reducing TSNAs as described above, thereby creating an additional benefit of such cigarettes, roll-your-own-tobacco, pipe tobacco, cigar filler

Claims

What is claimed is:

1. A cigarette comprising:

an increased-nicotine transgenic plant or plant portion of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion; and

a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method.

2. The cigarette according to claim 1, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion from which said transgenic plant or plant portion is produced.

3. The cigarette according to claim 1, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

4. The cigarette according to claim 3, wherein said enzyme is selected from a group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

5. The cigarette according to claim 1, wherein said plant species is *Nicotiana tabacum*.

6. The cigarette according to claim 5, wherein said yield is about 1 mg of tar and between about 0.12 mg and about 0.34 mg of nicotine.

7. The cigarette according to claim 5, wherein said yield is about 2 mg of tar and between about 0.25 mg and about 0.68 mg of nicotine.

8. The cigarette according to claim 5, wherein said yield is about 3 mg of tar and between about 0.36 mg and about 1.0 mg of nicotine.

9. The cigarette according to claim 5, wherein said yield is about 4 mg of tar and between about 0.50 mg and about 1.36 mg of nicotine.

10. The cigarette according to claim 5, wherein said yield is about 5 mg of tar and between about 0.62 mg and about 1.70 mg of nicotine.

11. The cigarette according to claim 5, wherein said yield is about 6 mg of tar and between about 0.75 mg and about 2.0 mg of nicotine.

12. The cigarette according to claim 5, wherein said yield is about 7 mg of tar and between about 0.87 mg and about 2.33 mg of nicotine.

13. The cigarette according to claim 5, wherein said yield is about 8 mg of tar and between about 1.0 mg and about 2.66 mg of nicotine.

14. The cigarette according to claim 1, wherein said tar-to-nicotine yield ratio is greater than about 3 and less than about 5.

15. A cigarette comprising an increased-nicotine transgenic plant or plant portion of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion.

16. The cigarette according to claim 15, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion from which said transgenic plant or plant portion is produced.

17. The cigarette according to claim 15, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

18. The cigarette according to claim 17, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

19. The cigarette according to claim 15, wherein said plant species is *Nicotiana tabacum*.

20. A method of making a cigarette comprising:

providing an increased-alkaloid transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*;

crossing said plant with a plant of the species *Nicotiana tabacum* to produce a progeny plant, wherein said progeny plant has an increased-nicotine phenotype; and

producing a cigarette comprising said progeny plant and having a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method.

21. The cigarette of claim 20.

22. The cigarette according to claim 21, wherein said transgenic plant species is *Nicotiana tabacum.*

23. A method of making a cigarette comprising:

providing a reduced-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of the species *Nicotiana tabacum*;

crossing said reduced-nicotine plant with a plant of the species *Nicotiana rustica* to obtain a progeny transgenic plant or plant portion; and

producing a cigarette comprising said progeny plant or plant portion.

24. The method according to claim 23, wherein said progeny plant or plant portion contains increased nicotine as compared to said reduced-nicotine plant or plant portion.

25. The method according to claim 23, wherein said progeny plant or plant portion contains decreased nicotine as compared to said *Nicotiana rustica* plant.

26. The cigarette of claim 23.

27. The cigarette according to claim 26 and having a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method.

28. A method of making a cigarette comprising:

providing an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion;

producing reconstituted tobacco from said plant or plant portion; and

producing a cigarette comprising said reconstituted tobacco.

29. The cigarette of claim 28.

30. The cigarette according to claim 29, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion from which said transgenic plant or plant portion is produced.

31. The cigarette according to claim 29, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

32. The cigarette according to claim 31, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

33. The cigarette according to claim 29 and having a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method

34. A method of making a cigarette comprising:

providing an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion;

producing expanded tobacco from said plant or plant portion; and

producing a cigarette comprising said expanded tobacco.

35. The cigarette of claim 34.

36. The cigarette according to claim 35, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion from which said transgenic plant or plant portion is produced.

37. The cigarette according to claim 35, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

38. The cigarette according to claim 37, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

39. A cigarette comprising:

a transgenic plant or plant portion of a species of the genus *Nicotiana* that exhibits increased nicotine as compared to a non-transformed control plant or plant portion from which said transgenic plant or plant portion is produced, wherein said transgenic plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion; and

a lower tar-to-nicotine yield ratio as compared to a control cigarette comprising said non-transformed control plant or plant portion.

40. The cigarette according to claim 39, wherein said increased-nicotine transgenic plant or plant portion, as compared to said non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as

compared to said non-transformed control plant and increased-nicotine content as compared to said non-transformed control plant or plant portion.

41. The cigarette according to claim 40, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

42. The cigarette according to claim 39, wherein said plant species is *Nicotiana tabacum*.

43. The cigarette of claim 39, wherein said tar-to-nicotine yield ratio is greater than about 3 and less than about 5.

44. A method of making a cigarette comprising:

providing an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion;

extracting nicotine from said transgenic plant or plant portion;

providing a plant or plant portion of a species of the genus *Nicotiana*;

adding said extracted nicotine to said plant or plant portion to form increased nicotine plant material;

producing a cigarette comprising said increased nicotine plant material.

45. The method according to claim 44, wherein said nicotine is nicotine salts of organic acids.

46. The cigarette of claim 44.

47. The cigarette according to claim 46, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion from which said transgenic plant or plant portion is produced.

48. The cigarette according to claim 46, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

49. The cigarette according to claim 48, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

50. The cigarette according to claim 46, wherein said plant species is *Nicotiana tabacum*.

51. The cigarette of claim 46 and having a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method.

52. The cigarette according to claim 51, wherein said yield is about 1 mg of tar and between about 0.12 mg and about 0.34 mg of nicotine.

53. The cigarette according to claim 51, wherein said yield is about 2 mg of tar and between about 0.25 mg and about 0.68 mg of nicotine.

54. The cigarette according to claim 51, wherein said yield is about 3 mg of tar and between about 0.36 mg and about 1.0 mg of nicotine.

55. The cigarette according to claim 51, wherein said yield is about 4 mg of tar and between about 0.50 mg and about 1.36 mg of nicotine.

56. The cigarette according to claim 51, wherein said yield is about 5 mg of tar and between about 0.62 mg and about 1.70 mg of nicotine.

57. The cigarette according to claim 51, wherein said yield is about 6 mg of tar and between about 0.75 mg and about 2.0 mg of nicotine.

58. The cigarette according to claim 51, wherein said yield is about 7 mg of tar and between about 0.87 mg and about 2.33 mg of nicotine.

59. The cigarette according to claim 51, wherein said yield is about 8 mg of tar and between about 1.0 mg and about 2.66 mg of nicotine.

60. The cigarette according to claim 51, wherein said yield ratio is greater than about 3 and less than about 5.

61. A method of making a cigarette comprising:

providing an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion;

extracting nicotine from said transgenic plant or plant portion;

providing an second plant or plant portion of a species of the genus *Nicotiana*;

adding said extracted nicotine to said second plant or plant portion to form increased nicotine plant material; and

producing a cigarette comprising said increased nicotine plant material and having a lower tar-to-nicotine yield ratio as compared to a control cigarette comprising said second plant or plant portion without the addition of said extracted nicotine.

62. The method according to claim 61, wherein said nicotine is nicotine salts of organic acids.

63. The cigarette of claim 61.

64. The cigarette according to claim 63, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion from which said transgenic plant or plant portion is produced.

65. The cigarette according to claim 63, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

66. The cigarette according to claim 65, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

67. The cigarette according to claim 63, wherein said plant species is *Nicotiana tabacum*.

68. A method of making a cigarette comprising:

providing a reduced-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that down-regulates the production of nicotine in said transgenic plant or plant portion;

producing cigarette tobacco from said transgenic plant or plant portion;

adding nicotine to said cigarette tobacco; and

producing a cigarette comprising said cigarette tobacco

said cigarette having a filler and said filler having a tobacco-specific nitrosamines level below about 0.5 micrograms per gram of said filler.

69. The method according to claim 68, wherein said nicotine is nicotine salts of organic acids, nicotine analogs or synthesized nicotine.

70. The cigarette of claim 68.

71. The cigarette according to claim 70, wherein said reduced-nicotine transgenic plant or plant portion exhibits reduced nicotine as compared to a non-transformed control plant or plant portion from which said transgenic plant or plant portion is produced.

72. The cigarette according to claims 70, wherein said reduced-nicotine transgenic plant or plant portion contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting reduced levels of said enzyme as compared to a non-transformed control plant or plant portion and reduced-nicotine content as compared to a non-transformed control plant or plant portion.

73. The cigarette according to claim 72, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

74. The cigarette according to claim 70, wherein said plant species is *Nicotiana tabacum.*

75. The cigarette according to claim 70, wherein said tobacco-specific nitrosamines level is less than about 0.05 micrograms (50ppb) per gram of tobacco.

76. The cigarette according to claim 70 and having a tar-to-nicotine yield ratio of between about 3 and about 8.

77. A method of making a tobacco product comprising:

providing a transgenic plant or plant portion of a species of the genus *Nicotiana* that exhibits reduced nicotine as compared to a non-transformed control plant or plant portion from which said transgenic plant or plant portion is produced, wherein said plant or plant portion expresses at least one heterologous nucleic acid that down-regulates the production of nicotine in said transgenic plant or plant portion;

producing tobacco from said transgenic plant or plant portion;

adding nicotine to said tobacco; and

producing a product comprising said tobacco and having a lower tobacco-specific nitrosamines level as compared to a control product comprising said non-transformed control plant or plant portion.

78. The method according to claim 77, wherein said nicotine is nicotine salts of organic acids, synthesized nicotine, nicotine analogs, non-nicotine alkaloids contained in any species of *Nicotiana*, and isomers of nicotine.

79. The tobacco product of claim 77.

80. The tobacco product according to claim 79, wherein said reduced-nicotine transgenic plant or plant portion contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting reduced levels of said enzyme as compared to a non-transformed control plant or plant portion and reduced-nicotine content as compared to a non-transformed control plant or plant portion.

81. The tobacco product according to claim 80, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

82. The tobacco product according to claim 79, wherein said plant species is *Nicotiana tabacum*.

83. A tobacco product according to claim 79, wherein said tobacco-specific nitrosamines level is below about 1 microgram per gram (1 ppm).

84. The tobacco product according to claim 79, wherein said tobacco product is in a form selected from a group consisting of leaf tobacco, shredded tobacco and cut tobacco.

85. The tobacco product according to claim 79, wherein said tobacco product is selected from a group consisting of snuff, pipe tobacco, cigar tobacco, chewing tobacco, cigarette tobacco, cigarette filler, lozenges, and any other nicotine delivery device other than a nicotine replacement product for nicotine replacement therapy.

86. A method of making expanded tobacco comprising:

providing an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion;

producing tobacco from said transgenic plant or plant portion; and

expanding said tobacco.

87. The expanded tobacco of claim 86.

88. The expanded tobacco of claim 87, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion from which said transgenic plant or plant portion is produced.

89. The expanded tobacco according to claim 87, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

90. The expanded tobacco according to claim 89, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

91. The expanded tobacco according to claim 87, wherein said plant species is *Nicotiana tabacum*.

92. A method of making reconstituted tobacco comprising:

providing plant material selected from a group consisting of (a) an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion and said cigarette, (b) a reduced-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that down-regulates

the production of nicotine in said transgenic plant or plant portion, (c) deproteinized tobacco fiber, and (d) freeze-dried tobacco; and

combining and reconstituting any combination of said plant material.

93. The reconstituted tobacco of claim 92.

94. A method of making reconstituted tobacco comprising:

providing a reduced-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that down-regulates the production of nicotine in said transgenic plant or plant portion; and

reconstituting said transgenic plant or plant portion.

95. The reconstituted tobacco of claim 94.

96. The reconstituted tobacco according to claim 95, wherein said reduced-nicotine transgenic plant or plant portion exhibits reduced nicotine as compared to a non-transformed control plant or plant portion.

97. The reconstituted tobacco according to claims 95, wherein said reduced-nicotine transgenic plant or plant portion contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting reduced levels of said enzyme as compared to a non-transformed control plant or plant portion and reduced-nicotine content as compared to a non-transformed control plant or plant portion.

98. The reconstituted tobacco according to claim 97, wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase

(PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

99. The reconstituted tobacco according to claim 95, wherein said plant species is selected from a group consisting of *Nicotiana tabacum* and *Nicotiana rustica*.

100. The reconstituted tobacco according to claim 95, and further comprising reconstituted deproteinized tobacco fiber.

101. A cigarette comprising the reconstituted tobacco of claim 95 and having a reduced yield of tobacco-specific nitrosamines (TSNA) as compared to a control cigarette comprising said non-transformed control plant or plant portion.

102. The cigarette according to claim 101, wherein said TSNA is selected from a group consisting of N'-nitrosonornicotine (NNN), 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butan-one (NNK), N'-nitrosoanatabine (NAT), and N'-nitrosoanabasine (NAB).

103. The cigarette according to claim 101, wherein said cigarette has a reduced yield of compounds selected from a group consisting of benzo(a)pyrene, phenols, and catechols as compared to a control cigarette comprising said non-transformed control plant or plant portion.

104. A method of making reconstituted tobacco comprising:

providing an increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, of a species of the genus *Nicotiana*, wherein said plant or plant portion expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion; and

reconstituting said plant or plant portion.

105. The reconstituted tobacco of claim 104.

106. The reconstituted tobacco of claim 105, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion.

107. The reconstituted tobacco according to claim 105, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

108. The reconstituted tobacco according to claim 107 wherein said enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

109. The reconstituted tobacco according to claim 105, wherein said plant species is *Nicotiana tabacum.*

110. The method according to claim 104, and further comprising freeze-drying said transgenic plant or plant portion after harvesting it.

111. A tobacco variety characterized by a high-nicotine trait and a high cured reducing sugar content, wherein said trait is conferred by a transgene.

112. A tobacco variety according to claim 111, wherein said variety is true-breeding for at least one of said high-nicotine trait and said sugar content.

113. A tobacco variety according to claim 111, wherein said sugar content ranges from between about 14 percent and about 30 percent.

114. A tobacco variety characterized by a high-nicotine trait and high fatty acid content, wherein said trait is conferred by a transgene.

115. A tobacco variety according to claim 114, wherein said variety is true-breeding for at least one of said high-nicotine trait and said fatty acid content.

116. A method for increasing nicotine and cured reducing sugar content in a *Nicotiana tabacum* plant, comprising:

transforming a plant having elevated cured reducing sugar content with a transgene conferring an increased nicotine phenotype;

regenerating progeny plants from said transformed plant; and

selecting a progeny plant having increased nicotine and increased cured reducing sugar content.

117. A method for increasing nicotine in a *Nicotiana tabacum* plant, comprising:

transforming a plant having elevated fatty acid synthesis with a transgene conferring an increased nicotine phenotype;

regenerating progeny plants from said transformed plant; and

selecting a progeny plant having increased nicotine and increased fatty acid levels.

118. A tobacco variety characterized by a high-nicotine trait and a cured reducing sugar content of at least 15 percent, wherein said trait is conferred by a transgene.

119. A tobacco variety according to claim 118, wherein said variety is true-breeding for at least one of said trait and said cured reducing sugar content.

120. A tobacco variety accord to claim 111, wherein said variety has a total alkaloid content of greater than 3.5 percent.

121. A tobacco variety characterized by a high-nicotine content of greater than 3.3 percent and a high cured reducing sugar trait, wherein said trait is conferred by a transgene.

122. A tobacco variety according to claim 121, wherein said variety is true-breeding for at least one of said sugar trait and said nicotine content.

123. A tobacco variety according to claim 121, wherein said cured reducing sugar content of said variety is greater than 15 percent.

124. A cigarette comprising:

an increased-nicotine transgenic plant or plant portion of a species of the genus *Nicotiana*;

a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method; and

a filler having a sugar to nicotine ratio of greater than about 3.5.

125. The cigarette according to claim 124, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion.

126. The cigarette according to claim 124, wherein said increased-nicotine transgenic plant or plant portion contains and expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion.

127. The cigarette according to claim 124, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

128. The cigarette according to claim 127, wherein said enzyme is selected from a group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

129. A cigarette comprising:

an increased-nicotine transgenic plant or plant portion of a species of the genus *Nicotiana*;

a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method; and

cigarette smoke having a pH of greater than about 6.

130. The cigarette according to claim 129, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion.

131. The cigarette according to claim 130, wherein said increased-nicotine transgenic plant or plant portion contains and expresses at least one heterologous nucleic acid that up-regulates the production of nicotine in said transgenic plant or plant portion.

132. The cigarette according to claim 129, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

133. The cigarette according to claim 132, wherein said enzyme is selected from a group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO),

NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

134. A cigarette comprising:

an increased-nicotine transgenic plant or plant portion of a species of the genus *Nicotiana*;

a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method;

cigarette smoke having a pH of greater than about 6; and

a filler having a sugar to nicotine ratio of greater than about 3.5.

135. A method of making a plant or plant cell culture, of a species of the genus *Nicotiana tabacum*, having an increase in the amount of a first alkaloid compared to the total amount of alkaloids comprising:

increasing the expression of a first enzyme selected from the group consisting of quinolate phosphoribosyl transferase (QPT) and putrescine N-methyltransferase (PMT) by introducing a nucleic acid encoding QPT or PMT into a plant cell;

altering, by increasing or decreasing, the expression of at least one additional enzyme involved in the biosynthesis or metabolism of one or more alkaloids or their precursors by introducing at least one nucleic acid into said plant cell; and

producing plants or plant cell cultures comprising said plant cell, wherein the amount of said first alkaloid compared to the total amount of alkaloids is greater in said *Nicotiana* plant or plant cell culture than the content of said first alkaloid compared to the total amount of alkaloids in a sibling control plant or plant cell culture of said *Nicotiana* plant in which only the expression of said first enzyme is increased.

136. The method of claim 135, wherein said first alkaloid is selected from the group consisting of nicotine cotinine, nornicotine, myosmine, nicotyrine, anabasine, anatabine, nicotine-n-oxide, N-methyl anatabine, N-methyl anabasine, pseudooxynicotine, 2,3 dipyridyl.

137. The method of claim 135, wherein said additional enzyme is selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), putrescine N-methyltransferase (PMT), quinolate phosphoribosyl transferase (QPT), and S-adenosyl-methionine synthetase (SAMS).

138. The method of claim 135, wherein said first alkaloid is produced by plants of a species of the genus *Nicotiana*.

139. The method according to claim 135, wherein said nucleic acids comprise at least one DNA sequence incorporated into a genome of said plant cell.

140. The method according to claim 135, wherein said nucleic acids is contained in a vector derived from a plant virus and is not incorporated into a genome of said plant cell.

141. The method according to claim 135, wherein said nucleic acids comprise sequences encoding all or part of said enzyme or the complements of said sequences.

142. The method according to claim 135, wherein said nucleic acids comprise sequences encoding all or part of a gene having a gene product that regulates the expression of a gene or genes encoding said enzyme or the complements of said sequences.

143. The method according to claim 135, wherein said nucleic acids comprise sequences selected from the group consisting of genes encoding transcription factors, genes encoding cyclophilins, a *nic1* gene and a *nic2* gene.

144. A cigarette comprising:

an increased-nicotine transgenic plant or plant portion of a species of the genus *Nicotiana*, wherein the production of nicotine in said plant or plant portion is up-regulated as a result of introduction of a synthetic or recombinant nucleic acid into said plant or plant portion; and

a tar-to-nicotine yield ratio of between about 3 and about 8, as measured by the FTC or ISO method.

145. The cigarette according to claim 144, wherein said increased-nicotine transgenic plant or plant portion exhibits increased nicotine as compared to a non-transformed control plant or plant portion.

146. The cigarette according to claim 144, wherein said increased-nicotine transgenic plant or plant portion, as compared to a non-transformed control plant or plant portion, contains and expresses at least one heterologous DNA encoding at least a segment of an enzyme required for the biosynthesis of nicotine in tobacco, said transgenic plant or plant portion exhibiting increased levels of said enzyme as compared to a non-transformed control plant or plant portion and increased-nicotine content as compared to a non-transformed control plant or plant portion.

147. The cigarette according to claim 146, wherein said enzyme is selected from a group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), ornithine decarboxylase (ODC), putrescine N-methyltransferase (PMTase), quinolate phosphoribosyl transferase (QPRTase), S-adenosylmethionine synthetase (SAMS), NADH dehydrogenase, and phosphoribosyl anthranilate isomerase (PRAI).

148. A method of making expanded tobacco comprising:
providing deproteinized tobacco;
expanding said tobacco; and
producing a cigarette comprising said tobacco.

149. The method according to claim 135, wherein said expression of said additional enzyme is altered by introducing a nucleic acid that induces a DNA repair mediated targeted mutation.

150. The method according to claim 135, wherein said expression of said additional enzyme is altered by introducing a molecular decoy.

151. The method according to claim 135, wherein said enzyme is selected from the group of enzymes produced by a plant of any species of the genus *Nicotiana* and involved in the biosynthesis or metabolism of alkaloids.

152. The method according to claim 135, wherein said first alkaloid is nicotine, said first enzyme is PMTase, and said additional enzyme is QPTase.

153. The method according to claim 152, wherein said nicotine is increased, said PMTase is increased, and said QPTase is decreased.

154. The method according to claim 152, and further comprising a second additional enzyme selected from the group consisting of arginine decarboxylase (ADC), methylputrescine oxidase (MPO), NADH dehydrogenase, ornithine decarboxylase (ODC), phosphoribosylanthranilate isomerase (PRAI), and S-adenosyl-methionine synthetase (SAMS).

155. The method according to claim 154, wherein said nicotine is increased, said PMTase is increased, said QPTase is decreased, and said second additional enzyme is decreased.

156. The method according to claim 154, wherein said nicotine is increased, said PMTase is increased, said QPTase is decreased, said second additional enzyme is decreased, and further comprising a third additional enzyme and said third additional enzyme is increased.

157. A tobacco plant according to claim 154.

158. The tobacco plant according to claim 157, wherein said tobacco plant has a nicotine to total alkaloid ratio greater than about 0.94 .

159. The tobacco plant according to claim 157, wherein said tobacco plant has a nicotine to total alkaloid ratio equal to or greater than about 0.98.

160. A tobacco product comprising the tobacco plant of claim 157, wherein said tobacco product is in a form selected from the group consisting of leaf tobacco, shredded tobacco and cut tobacco.

161. A tobacco product comprising the tobacco plant of claim 157, wherein said tobacco product is selected from the group consisting of snuff, pipe tobacco, cigar tobacco, chewing tobacco, cigarette tobacco, cigarette filler, lozenges, and a nicotine delivery device that is not a nicotine replacement product used for nicotine replacement therapy.

162. The tobacco product according to claim 161, wherein said tobacco product has a tobacco-specific nitrosamine level less than about 0.60 micrograms per gram of tobacco (600 ppb).

163. The tobacco product according to claim 162, wherein said tobacco-specific nitrosamine level is less than about 0.15 micrograms per gram of tobacco (150 ppb).

164. A filler of a cigarette comprising a tobacco plant according to claim 157.

165. A cigarette comprising a filler according to claim 164, wherein said cigarette has a tobacco-specific nitrosamine level less than about 0.20 micrograms per gram of tobacco (200 ppb).

166. A cigarette comprising a filler according to claim 164.

167. Seeds produced by a tobacco plant according to claim 157.

168. A method for increasing alkaloid biosynthesis in a *Nicotiana tabacum* plant or part thereof, comprising:

a) introducing into a plant cell of said plant:

(i) a first construct comprising, in the 5' to 3' direction, a promoter operably linked to a heterologous nucleic acid encoding quinolate phosphoribosyl transferase (QPT); and

(ii) a second construct comprising, in the 5' to 3' direction, a promoter operably linked to a heterologous nucleic acid encoding putrescine N-methyltransferase (PMT);

b) culturing said plant cell under conditions suitable for plant growth; and

c) selecting a progeny plant having increased alkaloid biosynthesis, wherein said progeny plant has increased QPT and PMT expression relative to a control plant.

169. A cigarette comprising said plant or part thereof according to claim 168.

170. A method for increasing nicotine biosynthesis in a *Nicotiana tabacum* plant or part thereof, comprising:

a) introducing into a plant cell of said plant

(i) a first construct comprising, in the 5' to 3' direction, a promoter operably linked to a heterologous nucleic acid encoding quinolate phosphoribosyl transferase (QPT); and

(ii) a second construct comprising, in the 5' to 3' direction, a promoter operably linked to a heterologous nucleic acid encoding putrescine N-methyltransferase (PMT);

b) culturing said plant cell under conditions suitable for plant growth; and

c) selecting a progeny plant having increased nicotine biosynthesis compared with a control plant.

171. A cigarette comprising said plant or part thereof according to claim 170.

172. A method for increasing alkaloid biosynthesis in a *Nicotiana tabacum* plant or part thereof, comprising:

a) introducing one or more nucleic acid sequences into a plant cell derived from said plant, wherein said nucleic acid increases quinolate phosphoribosyl transferase (QPT) and putrescine N-methyltransferase (PMT) expression;

b) culturing said plant cell under conditions suitable for plant growth; and

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c) selecting a progeny plant having increased alkaloid biosynthesis, wherein said progeny plant has increased QPT and PMT expression relative to a non-transformed control plant.

173. A cigarette comprising said plant or part thereof according to claim 172.

174. A *Nicotiana tabacum* plant variety having increased quinolate phosphoribosyl transferase (QPT) and putrescine N-methyltransferase (PMT) expression.

175. A cigarette comprising said plant or part thereof according to claim 174.

176. A method for increasing alkaloid biosynthesis in a *Nicotiana tabacum* plant or part thereof, comprising:

a) introducing one or more nucleic acid sequences into a plant cell derived from said plant, wherein said nucleic acid increases quinolate phosphoribosyl transferase (QPT) and putrescine N-methyltransferase (PMT) expression;

b) culturing said plant cell under conditions suitable for plant growth;

c) inducing progeny plants with a jasmonate; and

d) selecting a progeny plant having increased alkaloid biosynthesis, wherein said progeny plant has increased QPT and PMT expression relative to a non-transformed control plant.

177. A cigarette comprising said plant or part thereof according to claim 176.

178. A method for increasing alkaloid biosynthesis in a *Nicotiana tabacum* plant or part thereof, comprising:

a) introducing one or more nucleic acid sequences into a plant cell derived from said plant, wherein said nucleic acid increases quinolate phosphoribosyl transferase (QPT) and putrescine N-methyltransferase (PMT) expression;

b) culturing said plant cell under conditions suitable for plant growth;

c) inducing progeny plants with an auxin; and

d) selecting a progeny plant having increased alkaloid biosynthesis, wherein said progeny plant has increased QPT and PMT expression relative to a non-transformed control plant.

179. A cigarette comprising said plant or part thereof according to claim 178.

180. A method for increasing nicotine synthesis in a *Nicotiana tabacum* plant or part thereof, comprising:

a) introducing one or more nucleic acid sequences into a plant cell derived from said plant, wherein said nucleic acid up-regulates quinolate phosphoribosyl transferase (QPT) transcription;

b) culturing said plant cell under conditions suitable for plant growth; and

c) selecting a progeny plant having increased alkaloid biosynthesis, wherein said progeny plant has increased QPT transcription relative to a non-transformed control plant.

181. A cigarette comprising said plant or part thereof according to claim 180.

182. A method for increasing nicotine synthesis in a *Nicotiana tabacum* plant or part thereof, comprising:

a) introducing one or more nucleic acid sequences into a plant cell derived from said plant, wherein said nucleic acid up-regulates putrescine N-methyltransferase (PMT) transcription;

b) culturing said plant cell under conditions suitable for plant growth; and

c) selecting a progeny plant having increased alkaloid biosynthesis, wherein said progeny plant has increased PMT transcription relative to a non-transformed control plant.

183. A cigarette comprising said plant or part thereof according to claim 182.

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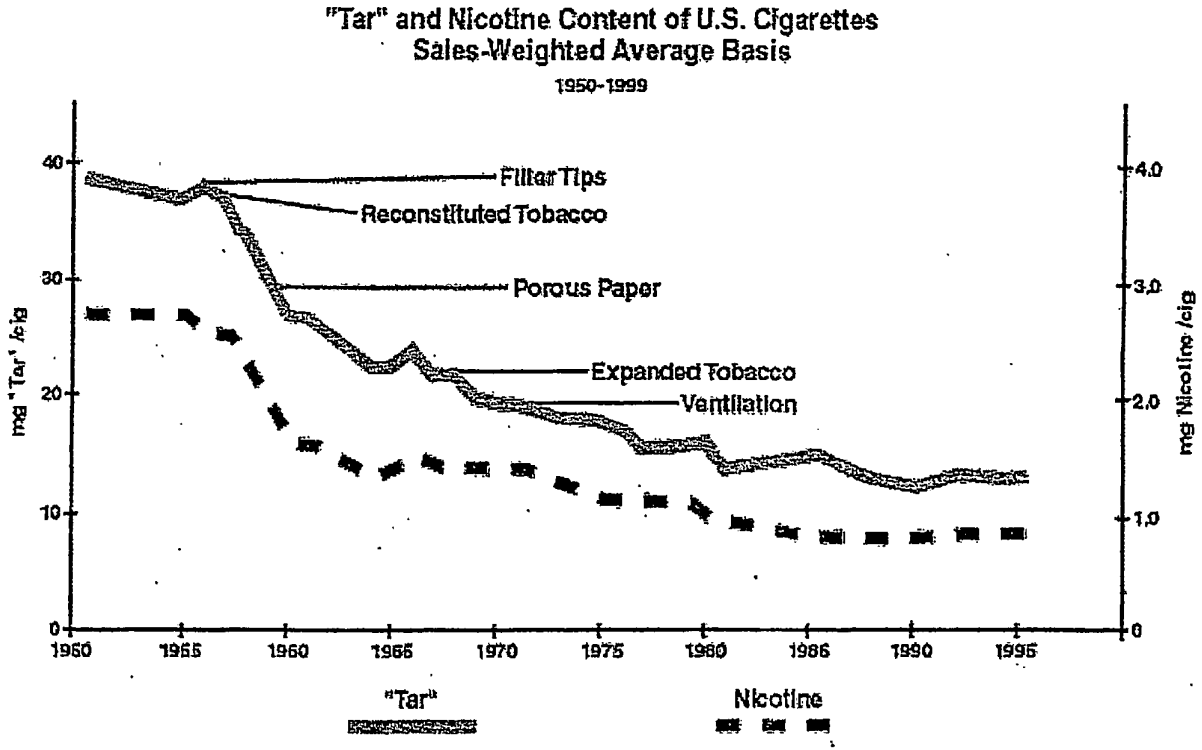
AMENDED SHEET

FIGURE 1

History of Tar & Nicotine Yields



Source: RJRT.com

- 1950 Sales-Weighted Average TNR (39/2.7) ≈ 14.44

FIGURE 2

Comparison of TNR's of Popular Brands*

	Tar	Nic.	TNR	
Marlboro Kings Filter Soft Pack	15	1.1	13.64	
Marlboro Medium Kings Filter Soft Pack	11	0.8	13.75	
Marlboro Lights Kings Filter Soft Pack	11	0.8	13.75	
Marlboro Ultra Lights Kings Filter Box	6	0.5	13.75	
Marlboro 100's Filter Box Red	15	1.1	13.75	
Basic Full Flavor Kings Filter Box	16	1	16.00	
Basic Lights Kings Filter Box	10	0.7	14.29	
Basic Ultra Lights Kings Filter Soft Pack	6	0.5	12.00	
Basic Full Flavor 100's Filter Soft Pack	16	1	16.00	
Virginia Slims Full Flavor 100's Filter Box	15	1.1	13.64	
Virginia Slims Lights 120's Filter Menthol Box	14	1.1	12.73	
GPC Full Flavor King	**15**	**0.8**	**18.75**	**(Highest)**
GPC Lights King Box	9	0.6	15.00	
GPC Ultra Lights King	5	0.4	12.50	
GPC Menthol Full Flavor 100	13	0.8	16.25	
Lucky Strike Non-Filter	23	1.5	15.33	
Lucky Strike Filter King Box	15	1.1	13.64	
Lucky Strike Lights King Box	**9**	**0.8**	**11.25**	**(Lowest)**
	Simple Average		**TNR 14.22**	

These brands represent about 50 % of U.S. market.

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FIGURE 3

Causes of Smoking-Related Deaths from 1990-1994 in U.S.

U.S. DHHS, 2000

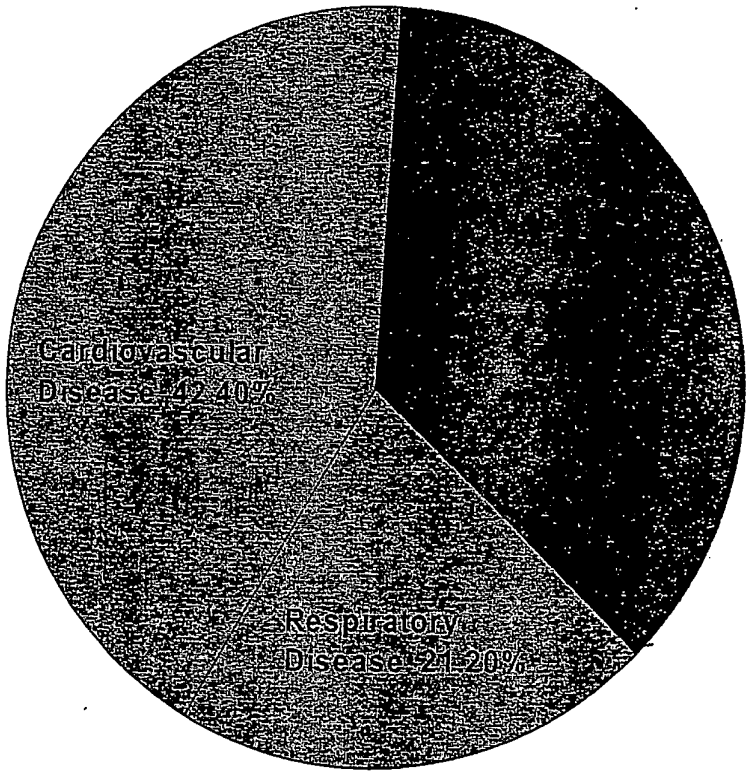

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FIGURE 5

Pathways of some Tobacco Alkaloids and 4 TSNAs

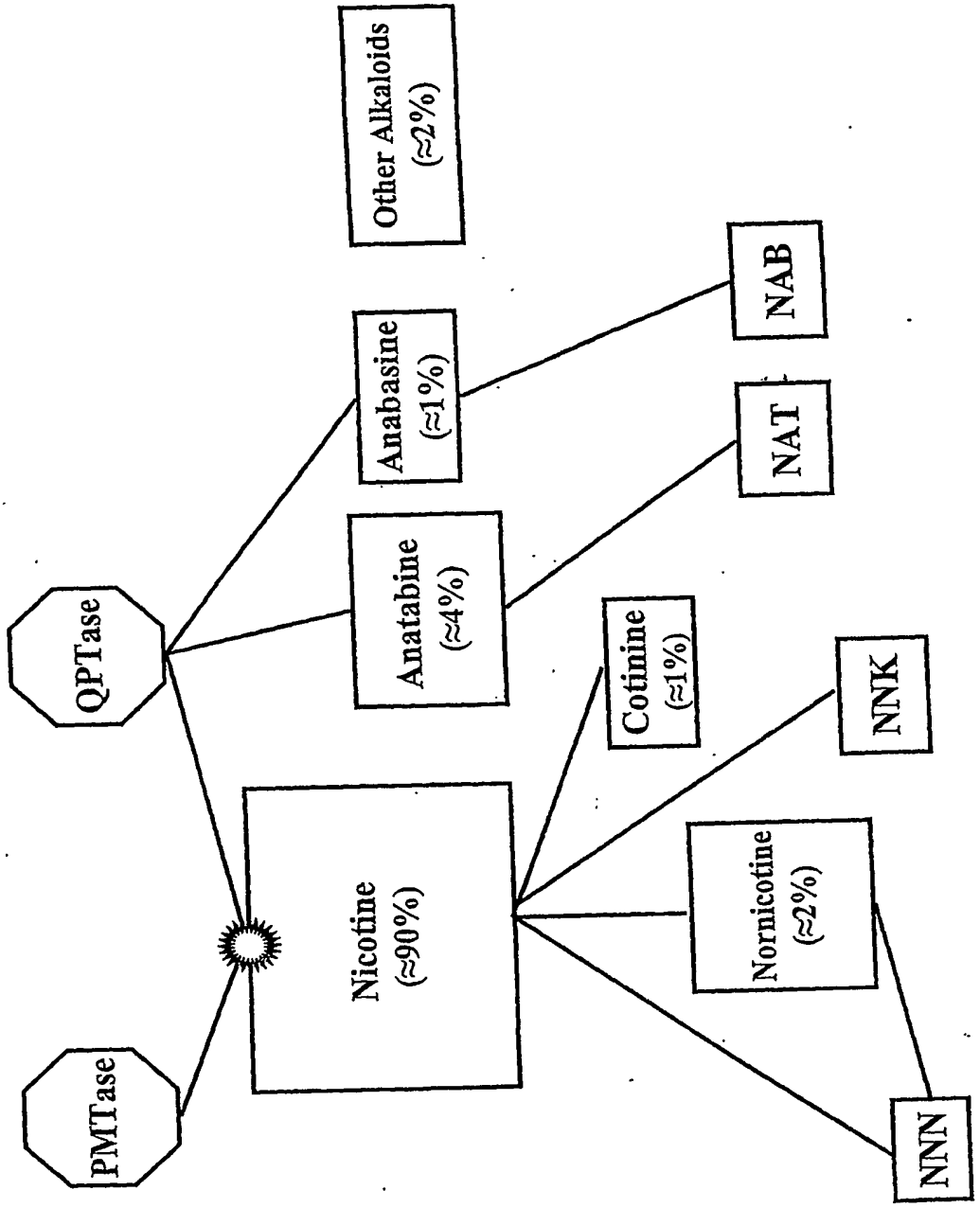

FIGURE 6

Schematic diagram of pyridine alkaloid biosynthesis in *Nicotiana*.

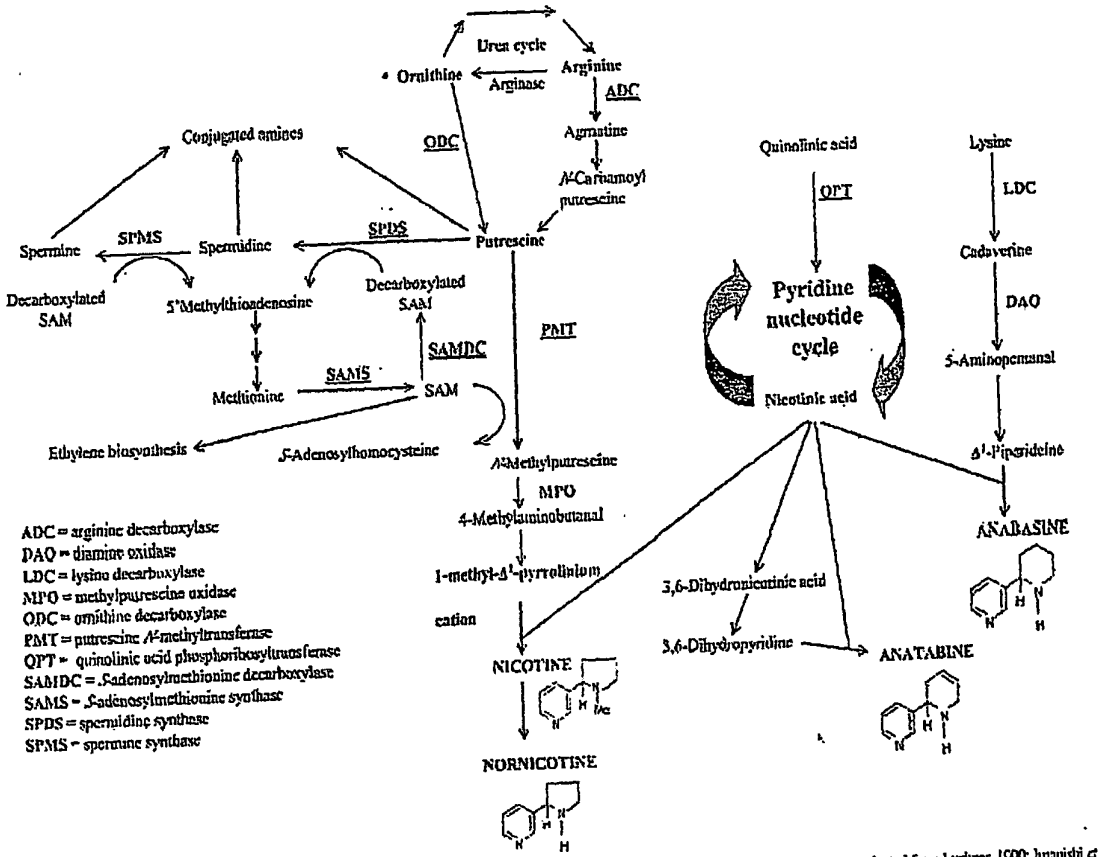

Figure 1. Schematic diagram of pyridine alkaloid biosynthesis in *Nicotiana* and its relationship with polyamine and pyridine nucleotide metabolism (adapted from Luckner, 1990; Imanishi *et al.*, 1998; Saito *et al.*, 2001). Enzymatic steps for which molecular probes were used for northern analysis in this study are underlined.

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